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TITLE: **Effects of Radiation on the Microbiota and Intestinal Inflammatory Disease**

PRINCIPAL INVESTIGATOR: **Stephen Shiao, MD, PhD**

CONTRACTING ORGANIZATION: **Cedars-Sinai Medical Center**
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6. AUTHOR(S)

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14. ABSTRACT

In this annual report (covering initiating and collaborating PI projects) we report the completion of experiments investigating the effect of inflammatory stimuli and focal irradiation of mice on the bacterial and fungal microbiota. We previously identified substantial changes in intestinal microbial communities induced by intestinal radiation exposure. Currently, we demonstrate that these changes correlate with increased sensitivity to inflammatory stimuli. As outlined in the project proposal, we are now in the midst of experiments aimed a evaluating the effects of radiation-induced changes in the microbiota on intestinal susceptibility to inflammatory disease.

15. SUBJECT TERMS

Radiation, microbiome, mycobiome, colitis, cancer

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1. INTRODUCTION

Exposure of the intestines to radiation may occur through unintended exposure from events such as nuclear accidents or through deliberate exposure to radiation such as during treatment for cancer. While a serious nuclear event might lead to many fatalities, an even larger number of people would be exposed to sublethal doses of radiation. These people, as well as patients who receive pelvic or abdominal radiation as part of their cancer treatment, often manifest bowel symptoms of diarrhea, and many people, even those with minimal acute symptoms, will develop long-term consequences of irradiation including permanent changes to bowel function and intestinal fibrosis, which can cause strictures or even bowel obstructions. It has been estimated that as many as 90% of patients receiving pelvic radiation experience long-term effects on gastrointestinal health, with over 50% reporting that the changes significantly degrade quality of life. The etiology of radiation-induced bowel toxicity has been linked to changes in the microvascular structure of the gastrointestinal tract, but increasing evidence suggests a role for immune cells associated with the intestine and their interactions with the normal microbial contents of the gut.

2. KEYWORDS

Radiation, microbiome, mycobiome, colitis, cancer.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Below is the Statement of Work (SOW) through the period of the previous and current annual review. Completion milestones are indicated.

Specific Aim 1: Define the alterations in gut microbiota (bacterial & fungal) in mice exposed to total body irradiation (TBI) or focal radiation to the GI tract.	**Timeline**	**Status**	**Site 1** (Stephen Shiao, MD, PhD)	**Site 2** (David Underhill, PhD)
Major Task 1: Effects of whole body radiation on bacterial and fungal microbiota.	Months			
Subtask 1: Expose mice (10/group) to <u>whole body</u>, <u>low dose</u> radiation & monitor weight loss & collect fecal pellets over 60 days. (40 animals)				
• Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens.	4-5	Completed (Jan. 2016)	Dr. Shiao	
• Evaluate bacterial/fungal diversity in all fecal samples.	5-6	Completed (Jun. 2016)		Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>whole body</u>, <u>high dose</u> radiation & monitor weight loss & collect fecal pellets over 60 days. (40 animals)				
• Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens.	5-6	Completed (Mar. 2016)	Dr. Shiao	
• Evaluate bacterial/fungal diversity in all fecal samples.	7-8	Completed (Aug. 2016)		Dr. Underhill
Subtask 3: Lock in fungal database & Train new staff.	1-3	Completed (Oct. 2015)		Dr. Underhill

Subtask 4: Expand repertoire of microbe-specific PCR primers to be used in the subsequent analyses. Train new staff.	1-3	Completed (Oct. 2015)	Dr. Shiao	
Local IRB/IACUC Approval	0	Completed (Aug. 2015)		
Milestone #1A: ACURO Approval.	4	Completed (Dec. 2015)	Dr. Shiao	
Milestone #1B: Database fixed and made available on website.	6	Completed (Nov. 2015)		Dr. Underhill
Major Task 2: Effects of focal radiation on bacterial and fungal microbiota.				
Subtask 1: Expose mice (10/group) to <u>abdominal</u>, <u>low dose</u> RT & monitor weight loss & collect fecal over 60 days. (40 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	6-7 7-8	Completed (May 2015) Completed (June 2015)	Dr. Shiao	Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>abdominal</u>, <u>high dose</u> RT & monitor weight loss & collect fecal over 60 days. (40 animals) • Perform radiation exposure, collect endpoint tissue for histological examination, evaluation of immune cell infiltration, PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	7-8 8-9	Completed (July 2015) Completed (Aug. 2015)	Dr. Shiao	Dr. Underhill
Milestone #2A: Complete processing & analysis of first 160 animals (effects of different types of radiation on the microbiome). Expect to find significant changes in bacterial, fungal, & immune parameters. *Milestone #2B: Co-author manuscript on the effects of radiation on the intestinal microbiota.*	12 10-16	Completed (July 2017) Ongoing	Dr. Shiao	Dr. Underhill
Specific Aim 2: Investigation of radiation-induced changes in sensitivity to a representative selection of murine models of intestinal inflammatory challenge.				
Major Task 1: Investigation of radiation-induced changes in sensitivity to DSS colitis				
Subtask 1: Expose mice (10/group) to <u>abdominal</u>, <u>low dose</u> RT & induce colitis with DSS. Monitor weight loss and collect fecal pellets for 12 days following exposure. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	9-10 10-11	Completed (Nov. 2016) Completed (Dec. 2016)	Dr. Shiao	Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>abdominal</u>, <u>high dose</u> RT & induce colitis with DSS. Monitor weight				

loss and collect fecal pellets for 12 days following exposure. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	11-12 12-13	Completed (Dec. 2016) Completed (Feb. 2017)	Dr. Shiao	 Dr. Underhill
Milestone #3A: Complete analysis of initial radiation-induced changes in DSS model. Expect to find significant changes in bacterial, fungal, & immune parameters.	16	Completed (April 2017)	Dr. Shiao	Dr. Underhill
Major Task 2: Investigation of radiation-induced changes in sensitivity to TNBS colitis & T cell transfer colitis				
Subtask 1: Expose mice (10/group) to abdominal, low dose RT & induce colitis with TNBS or $CD4^{+}CD45RB^{high}$ T cells. Monitor weight loss and collect fecal pellets over 12 days. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	13-14 14-15	Completed (Dec. 2016) Completed (Mar. 2017)	Dr. Shiao	 Dr. Underhill
Subtask 2: Expose mice (10/group) to abdominal, high dose RT & induce colitis with TNBS or $CD4^{+}CD45RB^{high}$ T cells. Monitor weight loss and collect fecal pellets over 12 days. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	15-16 16-17	Completed (April 2017) Completed (July 2017)	Dr. Shiao	 Dr. Underhill
Milestone #4A: Complete analysis of radiation-induced changes in colitis models. Expect to find significant changes in bacterial, fungal, & immune parameters.	24	Ongoing	Dr. Shiao	Dr. Underhill
Major Task 3: Investigation of radiation-induced changes in sensitivity to *S. typhimurium* & *C. albicans*				
Subtask 1: Expose mice (10/group) to abdominal, low dose RT & induce colitis with *Salmonella* or *Candida*. Monitor weight loss and collect fecal pellets for 12 days following exposure. (40 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	17-18 18-19	Completed (July 2017) Ongoing	Dr. Shiao	 Dr. Underhill
Subtask 2: Expose mice (10/group) abdominal, high dose RT & induce colitis with *Salmonella* or *Candida*. Monitor weight loss and collect fecal pellets for 12 days following exposure. (40 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens.	19-20 20-21	Completed (Aug 2017) Ongoing	Dr. Shiao	 Dr. Underhill

• Evaluate bacterial/fungal diversity in all fecal samples.				
Milestone #5A: Complete analysis of radiation-induced changes in colitis induced by infectious organism. Expect to find significant changes in bacterial, fungal, & immune parameters. *Milestone #5B: Co-author manuscript on the effects of radiation on sensitivity to intestinal inflammation as it relates to the intestinal microbiota.*	24 5-30	Ongoing Ongoing	Dr. Shiao	Dr. Underhill
Specific Aim 3: Manipulation of the intestinal microbiota to affect inflammation exacerbated by radiation exposure.				
Major Task 1: Effects of bacterial depletion on radiation-induced susceptibility to intestinal inflammation.				
Subtask 1: Deplete intestinal bacteria with antibiotics, expose mice (10/group) to abdominal RT as optimized above & induce colitis with DSS. Monitor weight loss & collect fecal pellets for 12 days. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	21-22 22-23	Completed (Aug 2017) Ongoing	Dr. Shiao	Dr. Underhill
Subtask 2: Deplete intestinal bacteria with antibiotics, expose mice (10/group) to abdominal RT & induce 2nd model of colitis as above. Monitor weight loss & collect fecal pellets for 12 days. (40 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	22-23 23-24	Ongoing Ongoing	Dr. Shiao	Dr. Underhill
Milestone #6A: Complete analysis of effects of bacterial depletion. Anticipate exacerbation of inflammatory parameters & large changes in fungal microbiome.	30	Ongoing	Dr. Shiao	Dr. Underhill
Major Task 2: Effects of fungal depletion on radiation-induced susceptibility to intestinal inflammation.				
Subtask 1: Deplete intestinal fungi with antifungal drugs, expose mice (10/group) to abdominal RT as optimized above & induce colitis with DSS. Monitor weight loss & collect fecal pellets for 12 days. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	23-24 24-25	Completed (Sept. 2017) Ongoing	Dr. Shiao	Dr. Underhill
Subtask 2: Deplete intestinal fungi with antifungal drugs, expose mice (10/group) to abdominal RT & induce 2nd model of colitis as above. Monitor weight loss & collect fecal pellets for 12 days. (40 animals)	24-25	Ongoing	Dr. Shiao	

• Collect endpoint tissue for histological examination, evaluation of immune cell infiltration, PCR of microbial burdens. Evaluate bacterial/fungal diversity in all fecal samples.	26-27	Ongoing		Dr. Underhill
Milestone #7A: Complete analysis of effects of fungal depletion. Anticipate exacerbation of inflammatory parameters & large changes in bacterial microbiome.	30	Ongoing	Dr. Shiao	Dr. Underhill

What was accomplished under these goals?

1) Major Activities

During this period from September 2016 – August 2017, **we completed both Major Task 1 and 2 for Specific Aim 2** as outlined in the statement of work (SOW). More specifically, we accomplished the following:

- We completed experiments comparing the effects of both high and low dose abdominal radiation on DSS induced inflammation (Major Task 1, Subtasks 1 and 2)

- We also completed experiments comparing the effects of both high and low dose focal abdominal radiation on TNBS-induced and T-cell mediated inflammation (Major Task 2, Subtasks 1 and 2)

- Analysis of bacterial and fungal microbiome changes in these inflammatory states have also been completed (Milestone #3A and #3B)

- We have also completed experiments comparing the effects of both high and low dose abdominal radiation on infectious inflammation (Major Task 3, Subtasks 1 and 2)

2) Specific Objectives

Following completion of our experiments in Specific Aim 1, we initiated the experiments outlined in Specific Aim 2. In a series of 4 large experiments (Specific Aim 2, Major Task 1, Subtasks 1 and 2), we compared two different doses of abdominal RT and the effects of the intestinal inflammation inducing agent DSS. We collected fecal samples throughout the course of the experiment to analyze the changes in the microbiome following the combination treatment. At the end of the experiment, we also harvested the intestines and mesenteric lymph nodes for multiparametric flow cytometry and histology to assess changes in the intestinal immune composition. We then completed an additional 8 experiments in which we compared two different doses of abdominal radiation with the alternative inflammatory agent TNBS and naïve T cells. Again, we collected fecal samples throughout the experiments and intestinal samples at the end of the experiment for assessment of changes in the microbiome and intestinal immune composition respectively (Major Task 2, Subtasks 1 and 2).

We then generated DNA from fecal samples collected throughout the experiment and analyzed then for overall bacterial and fungal content using quantitative PCR and sequenced the fecal samples to identify specific species. We are currently in the process analyzing the sequencing data in the context of the immune changes.

3) Significant Results/Key Outcomes

From our first set of experiments, we observed that following either high or low dose radiation to the abdomen that administration of DSS leads to increased weight loss in mive that had previously received RT (**Figure 1A**). This weight loss pattern was not mirrored in the oxazolone group though the mice treated with combined RT and oxazolone did exhibited a delayed recovery of weight compared to the oxazolone alone group (**Figure 1B**).

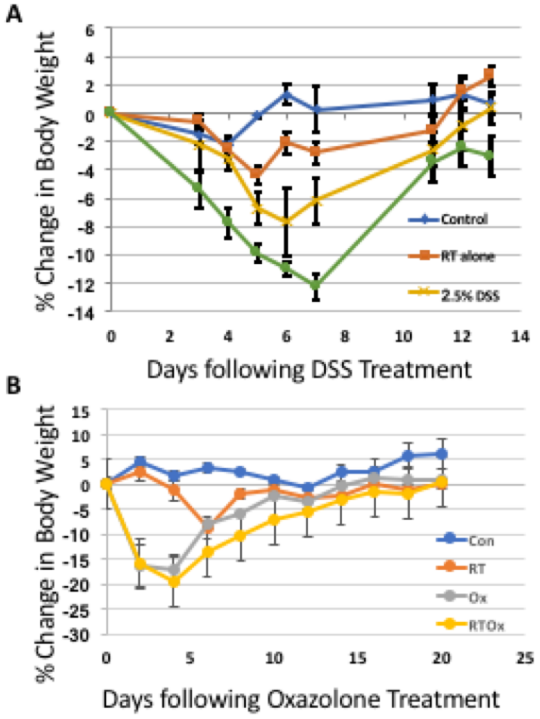


Figure 1. Abdominal RT induces greater sensitivity to the inflammatory stimuli DSS (upper) and Oxazolone (lower). N=8/group

We found that over the course of the experiments that RT prevented the shedding of bacteria and fungi in response to DSS into the feces (**Figure 2**). Previous sequencing data demonstrated that both TBI and abdominal RT produced marked changes in the populations of bacteria and fungi in the stool. Interestingly, RT appears to change the landscape such that different species become dominant rather than a global decrease in increase in all populations (**Figure 3**) and though RT seems to alter the microbiome landscape it also increases the amount of leak seen in the intestine suggesting that the increased inflammation observed may be attributed to both the microbiome changes and increased exposure to the altered microbiome components (Figure 4).

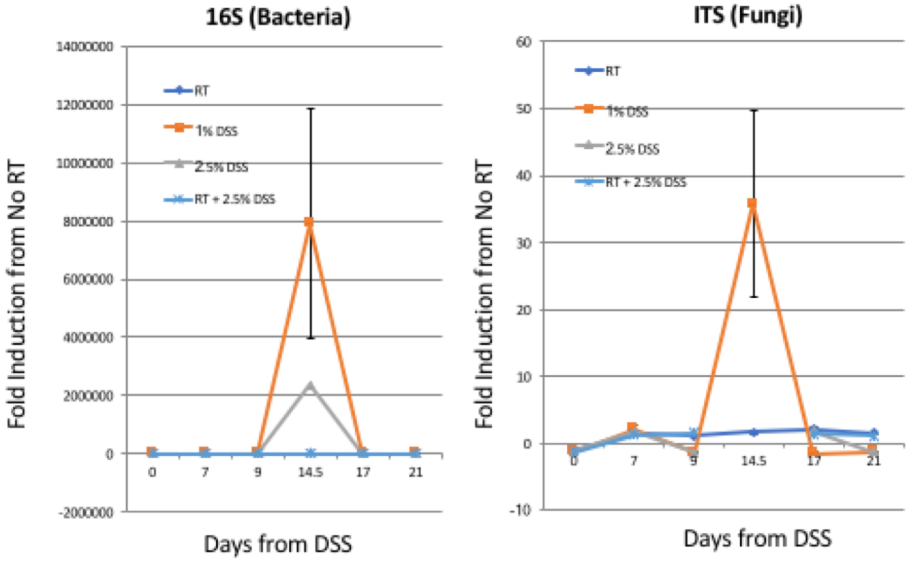


Figure 2. Abdominal RT and DSS prevent shedding of bacteria and fungi into the feces compared to DSS amd RT + DSS (6 mice/group)

Accompanying these changes in the micro- and mycobiome, we also found that there were significant changes in the $CD4^+$ and $CD8^+$ T cells, regulatory T cells, macrophages and dendritic cells in the mesenteric LN with RT and DSS (**Figure 4**). As observed in our earlier experiments, RT alone appears to decrease the overall number of immune cells, however the reduction is largely restricted to CD4+ and CD8+ T cells while leaving the CD11b+ macrophages, CD11c+ dendritic cells and

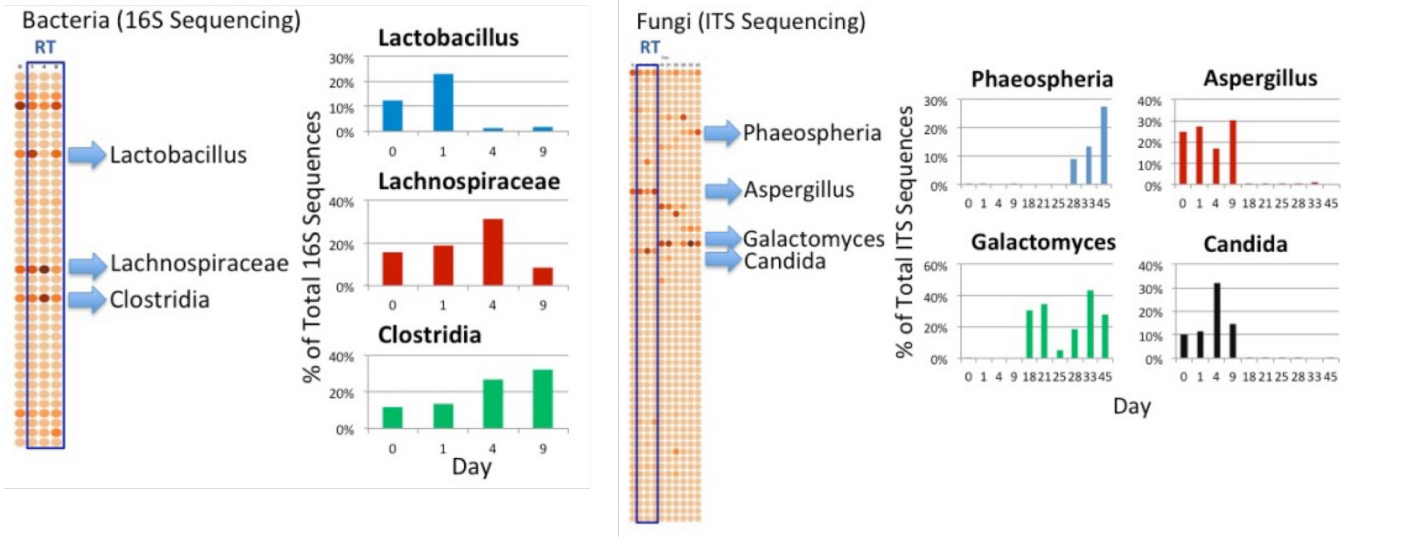


Figure 3. 16S and ITS sequencing show changes in both bacterial and fungal species following RT.

CD4+CD25+FoxP3+ regulatory T cells (Figure 5).

Though the analysis is currently ongoing, from our current set of experimental data, we conclude that abdominal RT leads to increased sensitivity to DSS. Given the significantly different effects on the bacterial and fungal populations in the intestine due to RT, we hypothesize that these changes lead to development of an increased inflammatory milieu such that administration of known gut inflammatory agents such as DSS and oxazolone. Further, we also find that there are significant effects of both DSS and oxazolone following RT on the immune changes in the mesentery likely in part due to the changes in the microbiome.

4) other achievements.

In addition to our experimental accomplishments, we continue to update the fungal database to include any new species of fungi we identified and post this database online for our other projects and for other groups to access.

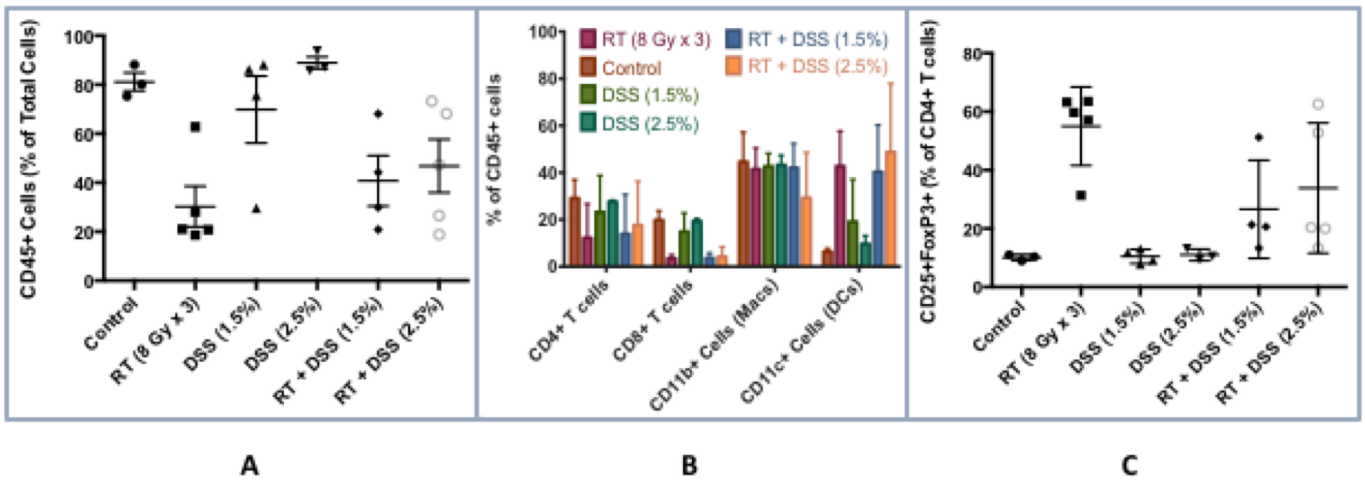


Figure 4. RT reduces the total number of CD45+ leukocytes (A) which holds true even with DSS. Reductions in CD4+ and CD8+ lymphocytes (B) and increases in CD11b+ Macrophages, CD11c+ dendritic cells and CD25+FoxP3+ regulatory T cells (B, C) were seen in both RT and RT/DSS groups. N=6-8/group.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

The project will continue as planned following the discussion in the text of the proposal and the experimental plans outlined in the Statement of Work. No substantial changes to this plan are currently anticipated.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

It is well-known that abdominal exposure to radiation often has intestinal consequences including diarrhea and intestinal inflammation and can lead to long-term disruption of normal bowel function and fibrosis. Less clear to date is the effect of radiation on the intestinal microbiome. A growing theme in our understanding of intestinal inflammation is that it is strongly dependent on the makeup of the microbiome and interactions of the host immune system with these organisms. Some prior human and animal studies had suggested that whole body radiation could affect intestinal bacterial populations. However, nothing has been known about how radiation exposure affects fungal communities in the gut, and nothing has been known about how radiation-induced changes in the microbiota may be associated with susceptibility to animal models of intestinal inflammatory disease.

As described in the outline of accomplishments above, in the first year of this project we have already made substantial new discoveries. Radiation exposure in mice results in profound changes in the fungal microbial population (as well as causing more modest changes in bacterial populations), and intestinal inflammation is exacerbated in both the DSS and oxazolone model of colitis.

What was the impact on other disciplines?

This project has supported the development and refinement of a unique manually curated fungal database. Characterization of microbiomes by high-throughput sequencing of microbial rDNA requires comparison of sequences recovered from a sample to a database linking those sequences to specific species of organisms. For bacteria, a long-standing effort has produced a well-accepted and commonly-used database of sequences. For fungi, this is more complicated and a “standard” database has not been available. We have generated a database used in this study that performs well at identifying fungal sequences in intestinal samples. It is expected that this database will be used widely by other groups in studies of intestinal microbiota as well as in studies of microbiomes at other sites. Further, we are elucidating a microbiome signature that predicts for increased inflammation which may be applicable across a wide range of intestinal inflammatory diseases.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Unfortunately, one of our postdoctoral fellows hired for this project has departed and we are currently in the process of hiring a replacement at this time. We anticipate only modest delay while we hire and train a new staff member.

Changes that had a significant impact on expenditures

The rate of expenditures has now caught up to the planned expenses and should continue on track to complete this project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS

Publications, conference papers, and presentations

Nothing to Report.

Website(s) or other Internet site(s).

https://risccweb.csmc.edu/microbiome/thf/
This is the publically-available download site for the fungal ITS "Targeted Host Fungi" (THF) database.

Technologies or techniques.

Nothing to Report.

Inventions, patent applications, and/or licenses.

Nothing to Report.

Other Products.

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

1) PDs/PIs.

Name:	*Stephen Shiao, M.D./Ph.D.*
Project Role:	*Initiating PI*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0001-7586-2885*
Nearest person month worked:	*Project #1: 2.5*

Contribution to Project:	*Dr. Shiao is the PI of project #1. He is responsible for overseeing all of the animal studies including radiation exposure, tissue harvesting, and immunophenotyping. He is responsible for managing all of the personnel participating in project #1.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	*David Underhill, Ph.D.*
Project Role:	*Collaborating PI*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0002-2989-658X*
Nearest person month worked:	*Project #2: 2*
Contribution to Project:	*Dr. Underhill is the PI of project #2. He is responsible for microbiome characterization in mouse tissue samples using high-throughput DNA sequencing of ribosomal genes. He is responsible for curating the fungal ITS database and for managing all of the personnel participating in project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

2) Other personnel.

Name:	*Jose Limon, PH.D.*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 6* *Project #2: 6*
Contribution to Project:	*Dr. Limon is a postdoctoral fellow working (50%) with Dr. Shiao on project #1 and (50%) with Dr. Underhill on project #2. He is performs the animal models of colitis and harvests tissue for analysis (project #1). He prepares DNAs and performs quality assurance test in preparation for sequencing of ribosomal DNAs (project #2).*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	*Paul Noe, B.S.*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 6*
Contribution to Project:	*Mr. Noe is a laboratory technician who has been involved in performing animal experiments in Project #1.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	*Viviana Maymi, B.S.*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*N/A*

Nearest person month worked:	*Project #1: 3*
Contribution to Project:	*Ms. Maymi is a laboratory technician who has been involved in performing animal experiments in Project #1.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Jie Tang, Ph.D.
Project Role:	*Genomics & Bioinformatics support*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 1*
Contribution to Project:	Dr. Tang is the acting director of the Cedars-Sinai Genomics core facility (replacing Dr. Vincent Funari), and has been instrumental in coordinating sequencing-based microbiome analyses in Project #2.
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Vineela Gangalapudi, Ph.D.
Project Role:	*Bioinformatician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 5*
Contribution to Project:	*Dr. Gangalapudi is a talented bioinformatician who has joined the Cedars-Sinai Genomics core to take the place of Dr Tang when he became director. She has been responsible for processing the high volume of sequencing data generated by project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Matthew Gargus
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 5*
Contribution to Project:	*Mr. Gargus is a laboratory technician who is responsible for processing samples for analysis in Project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Christian Leal
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	*N/A*

Nearest person month worked:	*Project #2: 1*
Contribution to Project:	*Mr. Leal was a laboratory technician who is contributed to processing samples for analysis in Project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Stephen Shiao, MD/PhD

Retired Support

Mann-Whitney-Eiger Award (Shiao) CTSI Scholar Seed Grant 09/01/14 – 09/01/15
"Influence of the Microbiome on the Efficacy of RT"
To examine the effect of the bacterial and fungal microbiome on the post-radiation anti-tumor immune response in a murine model of breast cancer.
Role: PI, 1% FTE, Total Direct+Indirect 1yr award: $30K
Grant Officer: Denis Magoffin (denis.magoffin@cshs.org)
No Overlap

Junior Faculty Award (Shiao) American Society of Radiation Oncology 7/1/14 – 6/30/2016 (currently no-cost extension (NCE))
"The Impact of Macrophage Polarization on the Efficacy of Radiation Therapy"
To define the effect of targeting macrophage bioeffector function in the anti-tumor immune response in a murine model of breast cancer.
Role: PI, 2.63% FTE, Total Direct+Indirect 2yr award: $200K
Grant Officer: Crystal Carter (research@astro.org)
No Overlap

Ongoing Support (No change or reduced effort)

K08 CA1191139 (Shiao) NIH/NCI 07/15/15 - 06/30/20
"The Impact of Macrophage Polarization on the Efficacy of Radiation Therapy"
To investigate the mechanisms of enhanced efficacy of radiation therapy with IL-4 blockade in a murine model of breast cancer.
Role: PI, 75% FTE, Total Direct+Indirect Requested 5yr award: $883K
Grant Officer: Susan Perkins (susan.ciolino@nih.gov)
No Overlap

David Underhill, Ph.D.

Retired Support

R21 AI103471 (Underhill) NIH/NIAID 2/1/2014 – 1/31/2016
"Measuring Phagosomal Temperatures"
To investigate the role of temperature in regulating formation and maturation of phagosomes in macrophages and dendritic cells.
Role: PI, 5% FTE, Total Direct+Indirect 2yr award: $422K
Grant Officer: Helen Quill (Hquill@niaid.nih.gov)
No Overlap

Senior Investigator Award (Underhill) Crohn's and Colitis Foundation 7/1/12 – 6/30/2015
"Anti-Fungal Immunity in Ulcerative Colitis"

To define the mycobiome in patients with ulcerative colitis and to explore associations with disease severity and functions of Dectin-1 polymorphisms.
Role: PI, 8% FTE, Total Direct+Indirect 3yr award: $347K
No Overlap

Ongoing Support (no change or reduced effort)

R01AI071116 (Underhill) NIH/NIAID 7/1/06 – 6/30/2018
"Dectin-1 Signaling Mechanisms"
To define the molecular and cellular mechanisms of signaling by the anti-fungal innate immune receptor Dectin-1.
Role: PI, 15% FTE, Total Direct+Indirect 4yr award: $1.8M
Grant Officer: Thomas Palker (palkert@niaid.nih.gov)
No Overlap.

R01 GM085796 (Underhill) NIH/NIGMS 4/1/12 – 3/31/2016 (currently no-cost extension (NCE))
"Innate Immune Sensing of Bacterial Sugars"
To define the innate immune mechanisms by which macrophages and dendritic cells detect bacterial cell walls.
Role: PI, 20% FTE (currently NCE reduced to 1%), Total Direct+Indirect 4yr award: $1.29M
Grant Officer: Sarah Dunsmore (dunsmores@nigms.gov)
No Overlap

R01 DK093426 (Underhill) NIH/NIDDK 7/1/12 – 6/30/2016 (currently no-cost extension (NCE))
"Host immunity to commensal gut fungi"
To define the roles of pathogenic fungi and the anti-fungal immunity genes for Dectin-1 and CARD9 in intestinal inflammation. There is no study of radiation in this project.
Role: PI, 15% FTE (currently NCE reduced to 1%), Total Direct+Indirect 4yr award: $1.78M
Grant Officer: Peter Perrin (Peter.Perrin@nih.hhs.gov)
No Overlap

PO1 DK046763 (Targan) NIH/NIDDK 9/2/16 – 8/31/2021
"IBD: Role of Genetic and Immunopathologic Mechanisms"
"Project 4: Immune Responses to Fungi Associated with Crohn's Disease (Project PI: Underhill)"
The project aims to understand the mechanisms of interaction of Crohn's disease-associated fungi *Malassezia* and *Aureobasidium* with the gut immune system.
Role: PI, 10% FTE, Total Direct+Indirect 5yr project 4 award: $2.1M
Grant Officer: Robert Karp (karpr@extra.niddk.nih.gov)
No Overlap.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

This is a collaborative award. Independent, but identical annual reports are filed. Contributions of each of the two projects and personnel have been indicated throughout the report.

QUAD CHARTS:

An updated quad chart has been included.

9. APPENDICES

1. Updated Quad Chart

Effects of radiation on the microbiota and intestinal inflammatory disease

Proposal No. PR140839, PR140839P1

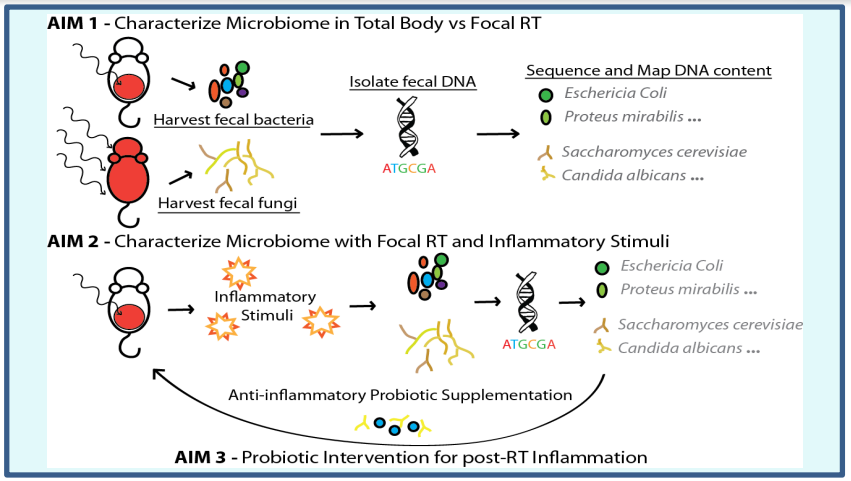
PI: Stephen Shiao MD PhD, David Underhill, PhD **Org:** Cedars-Sinai Medical Center **Award Amount:** $1,500,000.00

Study/Product Aim(s)

- **Aim 1:** Characterize the alterations in gut microbiota (bacterial & fungal) in mice exposed to total body irradiation (TBI) or focal radiation to the GI tract.
- **Aim 2:** Investigation of radiation-induced changes in sensitivity to a representative selection of murine models of intestinal inflammatory challenge.
- **Aim 3:** Manipulation of the intestinal microbiota to affect inflammation exacerbated by radiation exposure.

Approach

We will use immunohistochemistry, flow cytometry and next generation sequencing techniques in a murine model of gut irradiation to test the hypothesis that specific alterations in the microbial composition within the gut leads to increased sensitivity to inflammatory stimuli following intestinal exposure to radiation.



Accomplishment: Place a description of the latest scientific accomplishment here. Limit the comments to three lines or less to make them fit; be succinct. These comments are valuable since they show progress.

Timeline and Cost

Activities CY	15	16	17	18
Characterize changes in microbiome and gut immune cell composition in total body vs focal RT				
Delineate changes in microbiome and gut immune cell composition following RT and various inflammatory stimuli				
Investigate effect of altering the microbiome on the development of post-RT intestinal sensitivity				
Estimated Budget ($1.5 mi)	**$200K**	**$500K**	**$500K**	**$300K**

Updated: June 12, 2017

Goals/Milestones

CY15 Goal – Effects of total body irradiation vs focal RT on intestine
☑ACURO Approval, staff hired/trained (**COMPLETED**)
☑Fungal/Bacterial database available (**COMPLETED**)
CY16 Goals – Effects of total body irradiation (TBI) vs focal RT on intestine
☑Characterization TBI vs focal RT on bact/fung microbiota (**COMPLETED**)
☑ Analysis: microbiome changes in irradiated guts + DSS (**COMPLETED**)
CY17 Goal – RT-induced changes in gut sensitivity
❑Analysis of microbiome changes in irradiated guts in other colitis models and infectious organisms **(In Progress)**
❑Analysis of effects of bacterial/fungal depletion on gut sensitivity to RT
CY18 Goal – Intervention studies to alter RT-induced gut sensitivity
❑Analysis of effects of lactobacillus and saccharomyces supplementation on on gut sensitivity to radiation

Comments/Challenges/Issues/Concerns

- None

Budget Expenditure to Date (Shiao/Underhill)
Projected Expenditure: $562,500/$562,500 (Total $1,125,000)
Actual Expenditure: $571,800/$436,556 (Total $1,008,356)